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Memoirs of the Department of Agriculture in India

STUDIES IN DISEASES OF THE JUTE PLANT

(1) DIPLODIA CORCHORI SYD.

BY

F. J. F. SHAW, D. Sc. (Lond.), A.R.C.S., F.L.S
Second Imperial Mycologist

DIEU ET MON DROIT

AGRICULTURAL RESEARCH INSTITUTE, PUSA

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[Received for publication on 23rd January, 1920.]

The Disease in the Field.

During the summer of 1917[1] an area of about 40 acres on the Pusa Farm was placed under jute (*Corchorus capsularis*). The variety grown was "kakya bombai," a pure line race selected by the Fibre Expert to the Government of Bengal (Mr. R. S. Finlow) and possessing many advantages in size and yield over the races commonly grown by the ryot in the great jute-producing districts of Eastern Bengal. Not all the area under jute was sown at the same time, some being sown about the middle of March and the remainder not until June. As the season advanced, the superior height and thickness of stem of the early sown portion became very noticeable. In the case of jute grown for seed, however, the size of the stem is not of such importance as when it is grown for fibre.

By the beginning of August the early sown portion of the crop had reached a height of 10–12 feet, about twice the size of the late sown, and presented a dense and almost impenetrable growth. The plants were a bright green colour but throughout this portion of the crop several plants appeared to be drying up and wilting, with the formation of a dense black discoloured band round the stem at a point about 2–3 feet above the ground level (Pl. I, fig. 1). Such plants ultimately lost all their leaves and were left standing as dry black stems, forming relatively conspicuous objects among their healthy green neighbours. Intermediate stages in the progress of the disease showed that the blackening of the stem nearly always commenced with the formation of a discoloured ring or band

[1] *Scientific Reports of the Agricultural Research Institute, Pusa*, 1917-18, page 74.

round the stem which gradually increased in density and spread up and down the stem. Hence the suggested name of "black band" disease. Jute plants, as the crop is grown in the field, consist typically of a long straight stem which remains unbranched until near the apex where the flower-bearing axes arise. Such a stem has numerous lateral buds throughout its entire length and from these buds abortive branches often arise. These branches reach a length of 2–4 inches and then almost invariably cease growth and dry up; they remain as short brown twigs projecting from the main stem. It is noticeable in the field that the commencement of an infection is frequently from the base of one of these lateral twigs, where the dead tissues might be expected to afford an exceptionally favourable medium for the commencement of fungal growth. In other cases a leaf, or a lateral bud with leaves just open, is seen in a collapsed and blackened condition adhering to the main stem, and the disease appears to have spread from this point. As the disease extends on the main stem the bark splits longitudinally (Pl. 1, fig. 2) and the bast fibres are exposed. In the final stages the fibres can be seen brown and dry with the intervening tissues decayed away. Examination of the surface of a blackened stem showed the presence of minute spherical black bodies (Pl. 1, fig. 3), suggesting pycnidia, and frequently there were visible, even to the naked eye, small white aggregations, which appeared to be exuding from the spherical black bodies. On rubbing the hand up and down such a stem the fingers became covered with a black dust.

As the season advanced the number of infected plants rapidly increased until by the middle of October about 20 per cent. of the early sown crop was infected. The late sown crop remained small, about 6 feet high, and was surprisingly free from the disease—not one infected plant could be discovered. In addition to the jute seed crop at Pusa a quantity was being grown in neighbouring indigo factories for seed for the Fibre Expert. These crops all showed the same state of affairs as the Pusa crop. Where the jute had been sown early, and the plants were well developed and comparable in size to the early sown jute at Pusa, there the same disease was found; crops, however, which had been sown late, and were small in stature were free from the disease. A similar result followed from the inspection of the jute seed crop at more distant centres in Champaran.

In addition to the jute seed crop in Bihar, a further area was being grown for seed on an estate in Kamrup, Assam. The inspection of the Kamrup crop provided some very interesting information. On this estate jute (green *C. capsularis*) was extensively grown and the bulk of the crop was being cut for fibre, but an area, estimated to yield 1,000 maunds, was being kept for seed. In growing a crop for fibre the main consideration is to secure long straight unbranched stems which will give a good length of fibre. In growing a crop for seed the branching of the main

stem should be encouraged in order to increase the number of potential flower-bearing axes. Bearing these factors in mind the crop at Kamrup consisted of a thickly sown portion, in which the plants had grown up almost touching one another and which was being cut for fibre, and an area in which the plants had been thinned out to a distance of 18 inches and which was being kept for seed. All the jute was "kakya bombai" and in both cases the stems had reached a height of about 10 to 12 feet. In the case of the thinned out seed crop, however, the stems were rather thicker and of course more branched than in the fibre crop. This seed crop showed many cases of the disease in a fashion precisely similar to the Pusa crop. In the fibre crop, however, diseased individuals were much less numerous, and in fact the trouble would more or less have escaped notice and would not have been classed as a disease in this crop. A further correspondence with the facts observed in Pusa was furnished by two seed plots which had been sown on 21st June, a month later than the remaining seed and fibre crops. These plants were much smaller than the earlier sown plants and the disease was not established among them. In one corner of one of these plots, however, the plants had grown to an exceptionally large size, possibly owing to some local richness of soil, and here about 30 per cent. of the plants had the disease. The yield of seed from diseased plants is very greatly reduced, as such plants dry up quicker than their healthy companions and are liable to shed their seed in the field. The actual formation of seed in the capsules also appeared to be less than normal.

Thus the yield of seed per acre in the Assam plots was 5 maunds in 1917 in comparison with 7 maunds in 1916, and at Pusa the yield was 4·33 maunds in 1917.

In 1918[1] jute was again grown on the Pusa Farm but the incidence of the disease was much less, save in one field which had been under jute in 1916. Here the crop was so badly diseased that the whole area had to be cut and burnt. The fact that it is only stems of a certain size and maturity which are liable to infection was well illustrated by some statistics obtained from this crop. Of stems over 5 feet high a proportion of about 20 to 25 per cent. was infected and the same amount of diseased plants was observed on counting only stems which were 1 inch, or more, thick at the ground level. In any jute crop, however, there are a considerable number of plants which are the result of late germination and in which the stems remain thin and relatively short. Among stems of this size the disease was practically non-existent, and if such plants are included in the estimation the proportion of diseased stems may be as low as 3 per cent. The proportion of diseased stems among the larger plants, however, gives a more accurate measure of the extent of the damage to the crop.

[1] *Scientific Reports of the Agricultural Research Institute, Pusa*, 1918–19, page 69.

In Bihar, in 1918, the disease was generally much less than in the preceding year. This is probably to be attributed to the abnormal dryness (Plate VIII) of the air during September–October, when the disease is apt to spread most quickly. In all cases it was found that the late sown crop was relatively immune and the early sown, large, well grown crop was most liable to the disease.

In Eastern Bengal, in August–September, 1918, the disease was present in Dacca, Mymensingh, Sinjhani and Haldibari. The number of diseased stems, however, was very small, and unless the disease appears earlier it is evidently not likely to be a serious source of damage to the fibre crop. An interesting fact observed was that in Dacca red-stemmed varieties of *C. capsularis* appeared to be much less susceptible to attack than green-stemmed, while *C. olitorius* appeared to be quite free from the disease. In Rajshahi the jute crop was *C. olitorius* and here also the disease was practically absent.

In 1919 the condition of the jute crop on Dacca Farm by no means agreed with that in the previous season. The *olitorius* crop, both green and red-stemmed varieties, was attacked. The incidence of the disease was not heavy and varied considerably in different fields on the farm, the greatest damage seen in any one area was probably about 10 per cent. Red-stemmed *C. capsularis* was also attacked. These observations were quite sufficient to show that neither red-stemmed *capsularis* nor *olitorius* jute was resistant to the disease. At Chinsurah Farm the jute crop was *C. olitorius*, both red and green varieties being the same as at Dacca. The crop was in this case very fine, averaging about 14 feet in height, and there was not a single case of "black band" disease. At Rangpur Farm both the *olitorius* crop and green-stemmed *capsularis* were infected. The disease, however, only reaching an appreciable degree when the plants were of a certain size.

In Bihar, in 1919, the state of the jute crop was very similar to that in 1918. A considerable portion of the seed crop had, however, been sown as late as June and this was invariably clean and healthy. In the more early sown areas the crop was only slightly diseased and not to an extent which would seriously diminish the yield.

Reviewing these observations on the seed jute crop of the last three years certain general facts emerge :—

(1) The disease exists in Bihar, Assam and Bengal, and is evidently well diffused over the whole jute-bearing area.

(2) The incidence of the disease is in some way bound up with the crop reaching a certain degree of size.

Beyond these facts, however, the evidences as to the conditions which favour the increase of the disease are confusing and will be considered later.

The Cause of the Disease.

A microscopic examination of a diseased stem demonstrated the presence of a parasitic fungus. The small black spherical bodies visible just below the epidermis were the pycnidia of a *Diplodia*, and the black dust which comes off on handling a diseased stem was *Diplodia* spores. The small white aggregations which appear on the surface of a diseased stem are masses of immature hyaline *Diplodia* spores. The spores when mature are dark brown, bicellular bodies averaging 24 × 12μ within the limits 20–29μ × 10–15μ. The pycnidia were rounded black structures, about 200–300μ in diameter, with a conspicuous mouth (Pl. II, figs. 1, 2) : although numerous and almost touching they do not run together. This fungus is evidently identical with that previously described[1] by Sydow and Butler as *Diplodia Corchori* Syd. and first identified from material collected in 1910. Earlier than this the fungus had been collected, but not identified and named, from a wide area in Bengal including places as far as Kissenganj and Mymensingh. It has, however, never before 1917 been observed in such numbers as to suggest that it was anything but a stray parasite.

On splitting open the bark of a diseased stem the surface of the wood is found to be coloured a deep brown, and in advanced cases of disease is almost black. This discoloration is due to the presence of masses of dark brown hyphæ of *Diplodia* running over the surface of the wood. The fibre is similarly stained.

A transverse section of the stem shows that the pycnidia are very superficial and occur in the outer layers of the cortex just covered by a few layers of cork cells (Pl. II, fig. 1). The mouths of the pycnidia break through this cork covering. The hyphæ of the fungus ramify in the cortex and traverse the phloem in all directions ; they can frequently be seen following the course of a medullary ray as far as the cambium, where hyphæ are particularly numerous, and in fact this tissue seems to afford a peculiarly favourable medium for the growth of the fungus. This accounts for the discoloration of the outer surface of the wood in more advanced cases of disease. Hyphæ also penetrate into the wood and are easily visible in section in the cells of the xylem.

From diseased plants the spores of the fungus were obtained and germinated in pure culture. In culture the fungus forms a copious mycelial growth, the mature hyphæ being a dirty greyish colour merging into brownish black in the old cultures. Up to the present pycnidia have not been formed on artificial

[1] Sydow, H. et P., et Butler, E. J. "Fungi Indiæ Orientalis, Pars V." *Annales Mycologici*, Vol. XIV, 1916, page 196.

media but on the cellulose medium and on dead sterile jute stems papillate stromata were often produced.

In artificial inoculations the fungus sets up a very rapid degeneration in the tissues of the cortex and phloem (Pl. IV, figs. 1, 2). In sections of an early stage of an infection hyphæ can be seen ramifying in all directions in the cortex (Pl. IV, fig. 2; Pl. III, fig. 2), and it is evident that a cellulose dissolving enzyme is being secreted. The cellulose dissolving power of the fungus was tested in cultures upon pure cellulose. A cellulose (parchment) diffusion shell was placed in a flask with 50 c.c. of the following nutrient solution :—

	grm.
Ammonium phosphate	6
Potassium nitrate	6
Magnesium sulphate	1
Lactic acid	2
Water	1,000 c.c.

The interior of the diffusion shell was then infected with the mycelium and the flask placed in an incubator at 30°C. Within 24 hours hyphæ had grown through the diffusion membrane into the surrounding liquid. This penetration could only be the result of the solution of a part of the diffusion membrane by the hyphæ. After two months the fungus had formed a dense mycelial growth both within and without the diffusion shell, which had become quite soft and rotten. Such cellulose fibres as persist among the fungal hyphæ lose the characteristic colour reaction with Schultz solution which is given by the unaltered cellulose. No sugar could be detected in the liquid in the flask. A control flask, which had not been infected with the fungus, remained unchanged.

The fungus was then cultured upon pure cellulose, each culture containing about 0·5 gramme of cellulose and 50 c.c. of the following solution :—

	grm.
Potassium nitrate	10
Monocalcic phosphate	5
Magnesium sulphate	1
Water	1,000 c.c.

In this case the only carbon present in the medium was the cellulose. Three flasks were infected with the fungus and three were kept as controls. After two months, when vigorous growth in the infected flasks had nearly rotted the cellulose, the flasks were opened and the liquids filtered and made up to a volume of 100 c.c. From each liquid 25 c.c. were taken and then acidified with 11 c.c.

concentrated sulphuric acid and titrated with a solution of potassium permanganate (1 grm. in 1,000 c.c.). The liquids from the control flasks required respectively an average of 0·85, 0·75 and 0·85 c.c. of the potassium permanganate solution before a permanent colouration was obtained. For the entire liquid content of each control flask therefore approximately 3·2 c.c. of potassium permanganate solution were required. In the case of the three infected flasks 25 c.c. of the liquid required an average 2·3, 2·2 and 1·9 c.c. of potassium permanganate before a permanent coloration was obtained. For the entire liquid contents of an infected flask, therefore, an average of 8·4 c.c. of potassium permanganate solution was needed. Thus the liquid from the infected flasks had more organic matter in solution than that from the uninfected flasks, and this could only have come from the solution of the cellulose by the action of the fungus.

The power of setting up a rot in cellulose tissues does not, however, explain the manner in which the fungus gains entry into the stem of the host. This can only result from ingress either at some break in the superficial tissues or from direct penetration of the cuticle. An infection in which a minute piece of agar culture was placed on the surface of the stem resulted in direct penetration of the epidermis and cuticle (Pl. IV, fig. 2) within 12 hours (Experiment IX). There exists, however, in this case the possibility that the presence of a small piece of agar in contact with the stem may cause a local softening of the cuticle, rendering the passage of the fungus more easy than under natural conditions. The following experiment was, therefore, carried out with the object of deciding whether the fungus could penetrate the uninjured cuticle. Three glass rods terminating in a small funnel-shaped expansion were placed upright in the soil next to three jute plants so that the edge of each funnel was within 1 m.m. of a stem. A small piece of an agar culture of the fungus was then placed in the bottom of each funnel and the whole enclosed in a lamp chimney plugged with cotton wool. In the moist atmosphere the hyphæ grew out over the edge of the funnel and made contact with the jute stem. A dark stain appeared on the stem in the region of infection, but the plants showed no sign of wilting, and after a week the infections were opened and portions of the stem from the region of apparent infection were fixed for microscopic examination. Sections showed clearly that penetration of the stem had taken place, and scattered hyphæ, causing a local disintegration of the tissues, could be seen in the cortex (Pl. III, fig. 2). Unfortunately in this case cork formation had just commenced, and no cases could be seen in which hyphæ were directly penetrating the cork layers from the outside. The only place at which entry was obvious being in the region of a lenticel. The question, therefore, whether the hyphæ of *D. Cacaoi* can ordinarily penetrate the

uninjured cuticle and whether, if so, they do so by virtue of a special cutin-dissolving enzyme[1] or merely by mechanical pressure, as stated by recent investigators[2] in the case of other parasites, remains to be settled. It cannot be overlooked, however, that in the field the disease could spread rapidly even supposing that the epidermis of the host was impervious to the fungus. An easy point of entry is afforded by the numerous lateral branches which exist as small dry twigs, and it has already been mentioned that, in the field, a number of infections appear to originate in this way.

Inoculations.

Inoculations with pure cultures of *Diplodia Corchori* were first carried out during September, 1917. The plants used for the experiments were healthy jute stems, of the variety "kakya bombai," standing 7–10 feet high, along the eastern edge of the Pusa crop.

Experiment I. Three plants each received a small tangential cut on the stem surface, and the wound was infected with a small piece of agar culture of *D. Corchori*; two of the plants had the wound infections bound up with oiled paper. In each case a brown stain appeared at the seat of infection and spread up and down the stem; pycnidia and spores rapidly developed on the diseased tissues and when the discoloration had completely ringed the stem, the plant withered and lost its leaves. All the plants were dead in from 10 to 14 days after infection, the fungus spreading up and down the stems, which were left standing as blackened sticks.

Experiment II. Three plants were infected with pure cultures of *D. Corchori*, small portions of agar cultures being placed at the base of lateral shoots. These lateral shoots are quite small, about 1–3 inches long, and as a rule do not develop further, but are left on the mature plant as short dead twigs. In one of the plants the point of infection was loosely bound up with oiled paper. Four days after infection the lateral shoots were dead and black and a small brown stain was spreading in the axils of these shoots on each stem. In the case of the infection which had been bound up, the disease spread rapidly up and down and round the stem, as in Experiment I, and death took place about 14 days after infection. Pycnidia and spores of *Diplodia* were abundantly developed and the fungus was re-isolated in culture from these spores. Near the seat of infection, where the fungus was most strongly developed, the bark became cracked and the brown

[1] Whitelaw, S. P. "Infection and Immunity studies on the Apple and Pear Scab Fungi *Venturia inaequalis* & *V. pirina*." *Annals of Applied Biology*, Vol. 1, January, 1915.

[2] Blackman, V. H. & Welsford, E. J. "Studies in the Physiology of Parasitism." *Annals of Botany*, XXX, July, 1916.

Brown, W. "Studies in the Physiology of Parasitism." *Annals of Botany*, XXX, 1916.

Dey, P. K. "Studies in the Physiology of Parasitism." *Annals of Botany* XXVII, July, 1919.

discoloured surface of the wood could be seen. In the other two plants the fungus spread up and down one side of the stem but did not succeed in ringing it, and these plants retained their leaves as long as their healthy neighbours.

Experiment III. Three plants were infected with pure cultures of *D. Corchori*, each plant being wounded by means of a tangential cut on the stem surface. These wound infections were all left exposed to the air. Two of the plants died within 10 days, the other was not completely ringed by the disease and survived longer.

As the season was now far advanced and the crop was drying off, further inoculation work was stopped. These preliminary experiments had, however, shown that the fungus was capable of infecting both unwounded and wounded healthy jute stems. Inoculation experiments upon green-stemmed *C. capsularis* were resumed in 1918.

Experiment IV. Two pots were sown with jute on 12th March, 1918, and four young plants were infected from an agar culture of *D. Corchori* on 22nd April, 1918. The pot was kept under a large bell jar ; another pot sown at the same time, but not infected, was also kept under a bell jar. The plants in both infected and control pots lost their leaves, from being kept under a bell jar, in five days. The infections did not take.

Experiment V. Five plants, sown in a pot on 12th March, 1918, were infected with a young, 48 hour old, agar culture of *D. Corchori* on 2nd May, 1918. Four of the plants were infected at a leaf base and one on the stem. All the infected plants, and an equal number of uninfected plants were kept under bell jars. All the infected leaves wilted and fell off by 7th May, 1918, and in one case a black stain commenced to spread from an infected leaf base up the stem and a microscopic examination showed that *Diplodia* hyphæ were present. No stem damage was observed in the other case, and by 13th May, 1918, the plants, both infected and control, had become unhealthy from being kept under a bell jar. No definite conclusion as to whether the fungus could infect young jute stems could be drawn from these last two experiments.

Experiment VI. Two plants sown in a pot on 12th March, 1918, were infected from an agar culture, 48 hours old, of *D. Corchori* on 27th May, 1918. The length of stem within which the infection was done was enclosed in a glass lamp chimney, the ends of which were plugged with cotton wool. After 24 hours a brown stain was distinctly visible at the seat of each infection, and by 31st May the discoloration had spread and one plant was nearly ringed ; in both plants the leaves were yellowing and falling. Both plants were ringed by a black band by 3rd June, and *Diplodia* pycnidia and spores were clearly visible on the surface of the diseased tissues. One of the plants was completely wilted and dead by 6th June, and an

examination a week later showed that *Diplodia* hyphae had travelled through the cortex and reached the wood ; cortex and phloem were practically destroyed all round the stem for a stretch of several inches. The other plant remained green and healthy in its upper part, despite the fact that it also was completely ringed by the fungus at the seat of infection. An examination of the diseased section of the stem showed that the plant had reacted against the parasite and had formed new vascular tissue to one side of, and external to, the old tissue.

Experiment VII. Two plants, sown in a pot on 12th March, 1918, were infected from a young agar culture of *D. Corchori* on 3rd June, 1918. One plant was infected on the stem surface and the other at the base of an axillary shoot ; both infections were jacketed with lamp chimneys. A brown stain appeared at the seat of infection in 24 hours, and on 7th June had spread up and down and round the stems with the production of pycnidia. One plant died a few days later but the second survived with the formation of fresh vascular tissue as in the last experiment. Controls remained healthy.

Experiment VIII. Three plants, sown in a pot on 12th March, 1918, were infected from a young agar culture of *D. Corchori* on 7th June, 1918 ; all the infections were carried out on the uninjured stem surface and were jacketed with lamp chimneys. After 24 hours all infections had taken and a brown stain was spreading over about ½ inch of the stem at the seat of infection. Two of the infections were removed for microscopic work and the third was left standing, its glass jacket being removed on 10th June, 1918. This plant died with typical symptoms of *Diplodia* disease during the next week. A characteristic of the diseased stems in all inoculations, and one which has also been observed in the field, is the longitudinal splitting of the diseased bark by which the surface of the wood is laid bare. In some cases there appears to be an actual separation of the constituents of the bast fibre, and it may be inferred that the fungus has an action upon the tissues which is possibly analogous to that which takes place during retting. Controls remained healthy.

Experiment IX. Two plants, sown in a pot, on 12th March, 1918, were infected on 8th June, 1918, from a young culture of *D. Corchori*. These infections were made at 7 p.m. and were jacketed with lamp chimneys in the usual way. At 7 a.m. on 9th June, 1918, after 12 hours, the infections had taken and a small brown stain was spreading on the stem surface. Both these stems were removed, and the tissues fixed in chrome-acetic, for microscopic examination.

Experiment X. Four plants in pot culture, sown on 12th March, 1918, were infected on 25th July, 1918. These plants were about 6 feet high and 1 inch thick at the base of the stem ; the infections were jacketed with lamp chimney. None of the infections took. Small brown stains at the point of infection occurred

in two plants but they did not spread, and the plants remained living and healthy. This experiment took place during a period of relatively high temperature.

All the above inoculations (Experiments IV–X) were made from a series of agar cultures which had originated from a spore infection on agar in December, 1917. The possibility of the fungus declining in the virulence of its capacity to infect the living jute stem owing to prolonged growth in artificial culture could not be lost sight of, and a further series of infections from a fresh isolation of the fungus was carried out. The fungus was re-isolated from spores during a visit to Dacca in August, 1918, and infected upon living jute plants in the field. The inoculations were carried out on a small plot which was sown with jute on 5th March, 1918, and the plants subsequently thinned out to a distance of 18". This crop grew very well and attained a height of about 14 feet; at the time of these experiments it was just over flowering period.

Experiment XI. Infected five plants on 9th September, 1918, as follows:—

(1) A stem received a small tangential wound about 4 feet above ground level and was infected on the wound and the infection bound up with cloth. After 48 hours a brown stain was spreading from the seat of infection and pycnidia were produced and the plant ringed on 14th September, 1918. The plant wilted and died on 15th September, 1918.

(2) A stem was infected on a small tangential wound about 4 feet from the ground level, but the infection was not bound up and was left exposed to the air. The plant was killed by 17th September, 1918.

(3) Two stems were infected, each at the base of a small lateral shoot about 4 feet above ground level, and the infections bound up with cloth. One plant died on 17th September, 1918, but the other never became completely ringed and survived.

(4) One plant was infected at the base of a lateral shoot but the infection was not covered with cloth and remained exposed to the air. The infection spread with the usual symptoms but the plant was not completely ringed and did not die until 18th October. The plants killed in this experiment are shown in Pl. V, fig. 1.

Experiment XII. Infected four plants on 11th September, 1918, as follows:—.

(1) Two stems infected on tangential wounds and infections covered with cloth. Both these plants died—one on 19th September, 1918, and the other on 25th September, 1918.

(2) One stem infected on the stem surface and the infected section enclosed in a lamp chimney. This plant died on 25th September, 1918.

(3) One stem infected on a lateral shoot and enclosed in a glass lamp chimney. This infection took but did not ring the stem ; the plant remained healthy.

Experiment XIII. Infected five plants at the base of lateral shoots, and covered infections with cloth on 20th September, 1918. Four infections took—one plant was dead on 28th September, 1918, and three other plants died between 18th October, 1918, and 28th October, 1918. In this experiment out of five infections only one resulted in rapid death of the host. This result should be compared with the weather at the time of the infections (Pl. VIII) ; the influence of humidity and temperature upon the success or failure of inoculations is considered below with reference to some of the inoculations during 1919.

The following experiments were carried out in 1919.

Experiment XIV. In this experiment the inoculations were all done upon plants in pot culture. In each case the infection was carried out by placing a small piece of an actively growing agar culture of *D. Corchori* on the living stem and jacketing this section of the stem with a glass lamp chimney. The plants were grown from seed sown on 5th March, 1919.

(*a*) Two plants of green *C. capsularis*, two plants of red *C. capsularis* and two of red *C. olitorius* were infected on 27th June, 1919, at 10 a.m. The infections took upon the " kakya bombai ," producing a typical brown stain after 48 hours and ringing the stems by 10th July, 1919. Neither the red *capsularis* nor the *olitorius* was injured.

(*b*) Two plants of red *C. capsularis* and two of red *C. olitorius* were infected on 12th July, 1919, at 10 a.m. The infections upon red *capsularis* produced a brown stain on the stem in 48 hours ; the progress of the inoculations was exactly the same as on green *capsularis*. No result was obtained on red *C. olitorius*.

(*c*) Two plants of green *C. capsularis*, two of red *C. capsularis* and two of red *C. olitorius* were infected on 15th July, 1919, at 10 a.m. In each case one plant was infected upon the uninjured stem surface and the other in the axil of a small lateral shoot. All infections upon green and red *capsularis* took at once and one plant of each variety was dead by 22nd July (Pl. VI, fig. 1). In the case of the infections upon *C. olitorius* that in the axil of a lateral shoot set up a typical rot but did not succeed in ringing the stem and killing the plant.

Under the conditions of this experiment, therefore, the red *C. olitorius* seemed less easy to infect than either green or red *C. capsularis*.

Experiment XV. In this experiment the plants inoculated were growing in the field from seed sown on 5th March ; the infections were carried out upon the naked stem surface and were not jacketed in any way.

(*a*) Three plants of green *C. capsularis* were infected at 10 a.m. on 15th July in the axils of lateral shoots; two others were infected in a similar situation after making a small tangential cut on the stem surface and one was infected in a small cut at the base of the stem. All the wound infections and one of the infections upon the uninjured stem took and produced a brown rot at the seat of infection within 24 hours. These plants all died from 7–14 days after infection (Pl. V, fig. 2). In the remaining two infections on uninjured stems the inoculum was lost, probably washed away by rain, in the 24 hours succeeding the inoculation.

(*b*) Six plants of red *C. capsularis* and six plants of red *C. olitorius* were infected on 7th August at 11 a.m.; three plants of each variety were wounded. All wound infections took, with the usual symptoms of "black band," but in the case of the infections upon uninjured stems the inoculum in each case dried up and failed to infect the stem. The three wounded plants of red *capsularis* and one plant of red *olitorius* died 12 days after inoculation. In the remaining two wound infections on red *olitorius* the infection produced a black stain running up and down the stem, but did not succeed in ringing and killing the plant.

(*c*) Three plants of red *C. capsularis* and three plants of red *C. olitorius* were infected on the uninjured stem surface on 13th August at 10 a.m. The stem, at the seat of infection, was lightly covered with a small piece of cloth tied above and below the inoculum. Two of the infections upon red *capsularis* and one upon red *olitorius* succeeded in producing the typical discoloration on the stem but the plants were not ringed and did not succumb to the disease.

In this experiment, therefore, infections in the field were much less successful upon red *C. capsularis* and red *C. olitorius* than upon green *C. capsularis*. Under the conditions prevailing at the time of the inoculations the two red-stemmed varieties seemed less susceptible than the green-stemmed.

Experiment XVI. All the infections in this experiment were carried out on uninjured stems of green *C. capsularis* in the field; each infection was covered with a small strip of thin white cloth. Controls with sterile agar were set up in each case.

(*a*) On 23rd August at 11 a.m. 22 plants were infected. In only five cases were there any signs of the infection taking and only one plant died.

(*b*) On 28th August at 6 p.m. 12 plants were infected. All these infections took and 8 plants died. The first plant wilted on 4th September and 8 plants were dead by 26th September, when the experiment was closed.

(*c*) On 14th September at 11 a.m. 12 plants were infected. Nine plants were killed by the fungus, during the next three or four weeks.

(*d*) On 18th September at 4·30 p.m. 11 plants were infected. Six plants were killed by the fungus.

In this experiment, therefore, there were marked differences in the results with a series of identical infections. The controls remained healthy throughout.

Experiment XVII. On 14th September at 11 a.m. twelve plants of red-stemmed *C. olitorius* were infected. The infections were carried out upon the uninjured stem and were covered with small strips of cloth as in the previous experiment. Ten infections took, producing the typical "black band" on the stem. The experiment was closed before the plants were killed by the fungus.

Experiment XVIII. All infections in this experiment were carried out on late sown plants of green *capsularis*; the plants were grown in pot cultures from seed sown on 20th June.

(*a*) Three plants, each about 12″ high, were infected on 23rd July at 10 a.m. All infections were jacketed with lamp chimneys, and three controls, consisting of plants with a minute piece of sterile agar on the stem, were also jacketed. On 27th July one of the infected plants was dead, the remaining plants, both inoculated and controls, remained healthy.

(*b*) This experiment was a repetition of the last; the infections were made on 7 h August at 11 a.m. Of three plants infected one was killed by 4th August —the remaining infections did not take.

(*c*) On 25th July at 10 a.m. twelve plants were infected; these plants were *not* jacketed with lamp chimneys and the pots were standing in the open air on a verandah. One plant was killed by the fungus, the remaining 11 plants were not affected, the inoculum drying up.

(*d*) On 10th August at 10 a.m. these eleven plants were again infected. Six of the infected plants were killed by the 14th August and three more died by 23rd August (Pl. VI, fig. 2).

Factors in the Incidence of the Disease.

Evidence has clearly shown that *D. Corchori* has been widely diffused in Bihar, Bengal and Assam for many years past; therefore in the season 1917, when the disease was bad, there must have been some factors favourable to the appearance of "black band," which were not acting in 1918 or 1919, when the incidence of the disease was much less severe.

The analysis of the factors which produce any epidemic is a task of great difficulty, since of the numerous causes to be evaluated each has to be considered in relation to both parasite and host. Thus a disease may increase in virulence owing to some change in the host which renders it a more favourable medium for

the growth of the parasite or to a condition directly favouring the development of the parasite ; or both these factors may operate at once.

In dealing with a fungal disease one of the first factors to be considered is the variation between the climates of different years. It has already been mentioned that the disease develops most severely upon the mature plant, the crucial months being usually August and September. The principal features of the climate in these months in 1917, 1918, and 1919 are shown on Plates VII, VIII, and IX from which it appears that these months in 1917 in Bihar were generally more humid and cooler than the corresponding periods in 1918 and 1919. These two factors of temperature and humidity probably affected both parasite and host ; the weather of 1917 being in respect of its higher humidity more favourable to the fungus and delaying the ripening of the crop, thus giving the parasite more time to act. Such a correlation between humidity and disease is by no means uncommon. Thus the determining factor in the incidence of wheat rust in certain parts of India appears to be the atmospheric humidity during the early months of the year.[1]

The influence of climate on the incidence of " black band " disease was further emphasized by a study of the weather conditions during the inoculation experiments of 1919. In certain cases (Experiments XV*a* and XVI*b*, *c*, *d*) the percentage of successful infections was high, while in others (Experiment XVI*a*) the inoculations were a failure. It is suggested, by a comparison of the dates on which infections were made with the conditions of temperature and humidity prevailing at the time (Pl. IX), that the successful inoculations were those which coincided with a relatively high humidity, and that inoculations which failed were those carried out during a period of lower humidity and higher temperature. All these infections were carried out on " kakya bombai " in the field, and for a complete investigation of the influence of climate on infection a detailed record of humidity and temperature actually recorded in the jute field during a series of infections is required. It is not possible to give these data at present but Plates X and XI show a complete record for these conditions during Experiments XVI*a*. *b* and XVIII*c*. *d*, obtained from a hygrometer working in a laboratory within a short distance of the site of the experiments. A more numerous series of observations is needed to establish the relations between humidity and temperature, and the success or failure of inoculations. It may be recalled, however, that in certain cases the limits of humidity within which infections can occur have been proved to be relatively narrow. Thus it is stated [2] that infections of wheat with *Puccinia graminis tritici* do not succeed below a humidity of 95 per cent. at a

[1] Butler, E. J. " Fungi and Disease in Plants." Thacker, Spink & Co., Calcutta, 1918. p. 110.

[2] Lauritzen, J. L. " Relations of Temperature and Humidity to Infection by certain Fungi." *Phytopathology*, Vol. IX, Jan., 1919.

temperature of 68° F., and that the range of infection of bean (*Phaseolus vulgaris*) with *Colletotrichum Lindemuthianum* (Sacc. et Magn.) Biv. & Cav. lies between 92 per cent. and 100 per cent. at a temperature of 68° F. In the case of buck wheat (*Fagopyrum esculentum* Moench.) with *Isochyta Fagopyrum* the range of infection at 77° F. lies between 90 per cent. and 100 per cent.

The fact that late sown jute, and generally the smaller stems, escape the disease has been frequently mentioned and suggests a method of raising a clean seed crop. Of the cause of this relative immunity in late sown jute little can be said at present; that the immunity of late sown jute is not absolute is shown by the successful infections in Experiment XVIII. It is noteworthy that when from some local richness of soil late sown plants attain a large size they are frequently attacked by the disease. This suggests that the relative immunity of smaller stems may perhaps be due to some anatomical difference in the external layers which renders the smaller stems less liable to penetration by the germ tube of the fungus, or more probably that the development of a large stem is connected with some physiological condition which renders it a more favourable medium for the parasite. Other cases in which the host plant is more susceptible to the attack of a parasite when in the mature condition are not unknown. The susceptibility of *shaftal* (*Trifolium resupinatum*) to the attack of *Polythrincium Trifolii* in the Peshawar District being a case within the writer's experience. Chemical investigations are in progress on the composition of jute stems from late and early sown crops but are not, at present, sufficiently advanced to admit of any discussion. There is a suggestion, in the results obtained up to date, that the stems of late sown jute are richer in soda (Na_2O) and sulphuric acid (SO_4) than those of the early sown crop.

Field Experiments.

During the process of threshing out jute seed it was obvious that a large number of spores of *D. Corchori* would become mixed with the seed and might serve to disseminate the disease in the next season's crop. Microscopic examination of samples of jute seed from a badly diseased crop showed the presence of *Diplodia* spores among the seed; the fact, however, that *D. Corchori* was already present in the jute-growing districts suggested that the presence of spores mingled with the seed would not prove a very potent factor in increasing the amount of disease. As, however, the Bihar seed crop was to be distributed throughout the jute-growing districts of Bengal in small packets, it was considered advisable to disinfect the seed, pending the results of experiments designed to show whether seed disinfection had any influence in lessening the incidence of the parasite. Experiments showed that jute seed could be steeped for 10 minutes in a 2 per cent. solution of copper sulphate and thoroughly dried without injury to germination, and that this treatment would

inhibit the germination of the spores of *D. Corchori*. In 1918, and in 1919, therefore, the whole of the jute seed crop of Bihar was disinfected in this manner before despatch to Bengal, and in these years the experiments detailed below were carried out in Pusa and the vicinity to test whether this treatment had any effect on the incidence of the disease.

In 1918 the following field experiments were made :—

Plots A and B. Two plots, each about $\frac{1}{10}$th of an acre, were sown on 5th March with jute seed ("kakya bombai") obtained from the diseased Pusa crop of 1917. This seed had not been treated by steeping in copper sulphate solution, but the land had not been under jute for over 20 years and no other jute was in the vicinity. One plot (A) was thinned out to a distance of 18" between plants, the other plot (B) was not thinned. Both plots gave a good crop. In the crop which had been thinned out the plants reached a height of 14 feet and a thickness of 1–1$\frac{1}{2}$ inches, but in the crop which was not thinned the plants only reached a height of 8 feet and the stems were much thinner. The plots were kept under observation throughout the season, and were cut and harvested on 5th November; the *Diplodia* disease was practically absent, only some half dozen infected plants occurring in each plot.

Plots C and D. These were a repetition of the two previous plots, Plot C being thinned out, and gave the same result.

From these experiments in which disease-free soil was sown with untreated seed, and in which the disease failed to appear to any appreciable extent, it may be inferred that the spread of the disease through spores mingled with the seed is not very serious.

Plots E and F. Two plots, each about $\frac{1}{4}$th acre, were sown with treated jute seed ("kakya bombai") on 1st March. The plots were situated in a portion of the field in which jute had been grown during the previous season and where the disease had been particularly bad. Unfortunately owing to deficiency of moisture during March and April, the seed did not germinate and the plots had to be resown at the end of April. Germination when it did take place was late and the crop in size and appearance resembled the ordinary late sown crop. Owing also to irregularity in germination in both plots the plants were fairly widely separated and the original intention of thinning out one plot was useless. Both these plots showed more *Diplodia* disease than the previous plots. Thus Plot E had 67 plants infected with *Diplodia* and Plot F had 54 plants infected with *Diplodia*.

In this experiment, therefore, a crop, which was virtually a late sown crop grown from treated seed, developed the disease when grown in land which had carried diseased jute during the previous season.

Plot G. This plot, about $\frac{1}{4}$th acre, was situated outside Pusa in land which had not been under jute for many years and which had no other jute near it. The crop was grown from treated seed ("kakya bombai") sown on 11th March. The plot was about $\frac{1}{4}$ acre in size and germination was at first uneven owing to deficiency of moisture. The crop did not reach a good height but was fairly thick in the stem; one end suffered considerably from flooding. There were about 60 cases of *Diplodia* in this crop.

In 1919 a further series of field experiments was made.

Plots A and C. These plots were sown with seed of "kakya bombai" about 5th March, the former with treated and the latter with untreated seed. *D. Corchori* was practically absent in both these plots, only some 3 or 4 cases could be seen. A crop of *Corchorus olitorius* in Plot B and one of red-stemmed *C. capsularis* in Plot D also remained free from the disease.

Plots E and F. These two plots from last season's experiment were again sown with jute of the variety "kakya bombai," the seed used had been steeped in a solution of copper sulphate, and this land had been under jute since 1917. In 1919, therefore, germination was very scanty. Both plots were resown on 4th July, after the commencement of the rains, and gave a crop of typical late sown jute, short in height and thin in stem. In both plots the number of stems infected with *D. Corchori* was negligible, only about 12 cases could be found when the crop was cut early in November. Thus in these plots the disease was less in 1919 than in the previous season.

Plots H and K. About $\frac{1}{3}$rd of each plot was sown on 5th March with a red-stemmed variety of *Corchorus capsularis* and the remainder with "kakya bombai." These plots were situated in the land which had carried the diseased jute in 1918. Both the varieties of seed sown had been steeped in 2 per cent. copper sulphate. Plot H carried a very scanty crop and had 31 cases of *D. Corchori* among the "kakya bombai" stems and only 6 cases in the red-stemmed variety. In plot K the crop was much thicker, both germination and growth having been better than in plot H. In Plot K there were 190 cases of *D. Corchori* among the "kakya bombai" stems and 34 cases in the red-stemmed variety.

In both these plots, allowing for the larger proportion sown with "kakya bombai," the red-stemmed variety suffered less than the green-stemmed. This result agrees with the result from infections upon red and green-stemmed varieties of jute (*see* Exp. XV), but at the same time it must not be lost sight of that the disease can infect red-stemmed jute in the field as is shown by the record at Dacca in 1919.

Plots M and N. These plots, each about $\frac{1}{4}$th acre, were selected in good land which had never carried jute before. Plot M was sown on 5th March with seed of "kakya bombai" which had been treated by steeping in copper sulphate solution, and Plot N was sown on the same date with seed which had not been so treated. Both plots gave an excellent crop of jute 9–11 feet in height. In both plots nearly the same number of stems were diseased owing to *D. Corchori*—76 stems in Plot M and 56 in Plot N.

As a result of these field experiments, particularly from a consideration of Plots M and N in 1919, it cannot be said that seed steeping in a solution of copper sulphate has any influence on the severity of the disease, and, therefore, as mentioned above, the dissemination of the disease cannot take place to any appreciable extent through spores of *D. Corchori* mingled with the jute seed. The percentage of disease was also not to any extent greater in those plots which had been under jute for two or more successive seasons.

Conclusions.

The present investigation has shown that—

(1) *Diplodia Corchori* Syd. is a parasite of the jute plant.

(2) The disease occurs after flowering and threatens the seed crop.

(3) The fungus is widely distributed in jute-growing districts.

(4) The intensity of the disease varies greatly from one season to another.

(5) The disease is most severe on large, well-grown stems, and infection takes place more readily upon green-stemmed than upon red-stemmed varieties.

Further research is required to show the precise mode of infection, the limits of temperature and humidity under which infection will take place, and the qualities which render the late sown crop resistant to the disease. Direct treatment against a disease such as this is scarcely possible in the case of the jute crop, and we must look to an increased knowledge of the factors which condition success in the life of the parasite, and to the possibility of modifying these factors by alterations in the culture of the host, for the effective control of this disease.

Any disease which threatens the jute plant might, in view of the importance of this crop in the economic life of Bengal, become a factor of grave agricultural importance. It is a matter of congratulation that the crop is generally free from fungal disease and that the parasite, which forms the

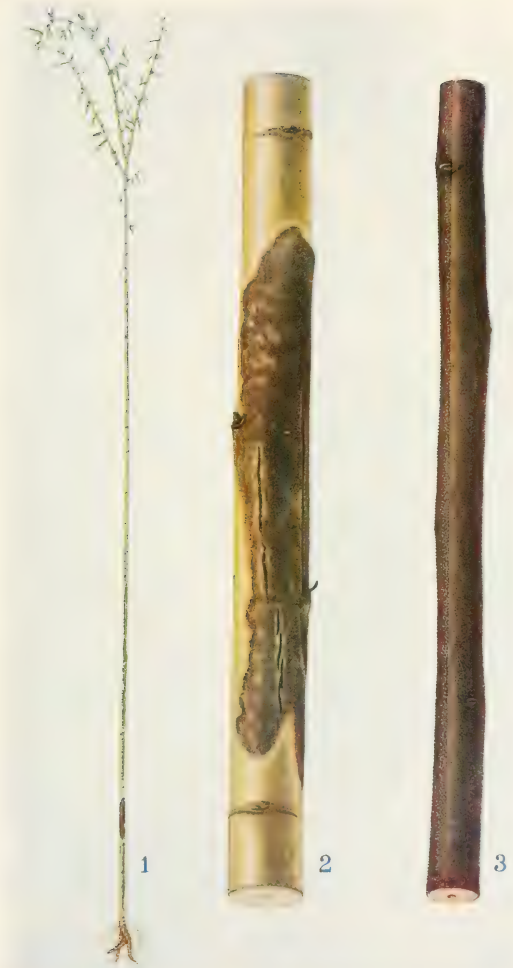
subject of the present paper, is not a source of danger to the fibre crop. Another stem rot of the jute plant is caused by a fungus, which has been identified[1] in Japan as *Macrophoma Corchori*[2] Saw. This fungus occurs in India,[3] where its depredations are not confined to the jute crop, and will form matter for a later communication.

[1] Kaneyoshi Sawada. "Preliminary Report of a new Stem Rot Disease of Jute caused by *Macrophoma Corchori* Saw. sp. nov." *Bull.* 107 *Agric. Expt. Sta. Formosa*, 1916.

[2] *Mycologia*, Vol. XI, No. 2, March, 1919, p. 82.

[3] *Scientific Reports of the Agricultural Research Institute, Pusa*, 1918-19, p. 71.

LIST OF ILLUSTRATIONS.



1, 2. A Jute plant showing Commencement of infection of *D. Corchori*.
3. Final stage of disease.

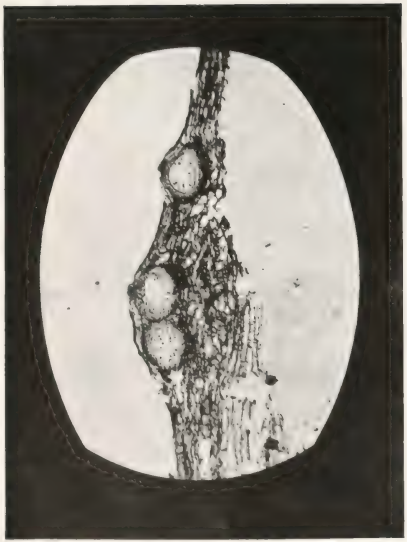


Fig. 1. Microphotograph of a section of the cortex a diseased stem showing three pycnidia of *D. Corchori* ×90.

Fig. 2. Pycnidium of *D. Corchori* ×500.

PLATE II.

Fig. 1. Germinating spores of *D. Corchori* (×500).

Fig. 2. Section of a cortex showing a hypha of *D. Corchori* setting up disintegration in the cells (×600).

PLATE III

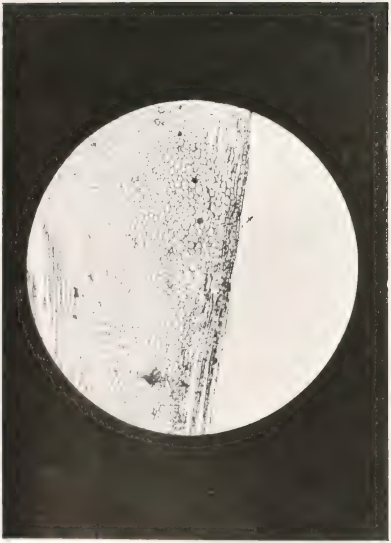


Fig. 1. Section of the cortex showing an early stage in an infection. The diseased tissue is visible between the marks xx.

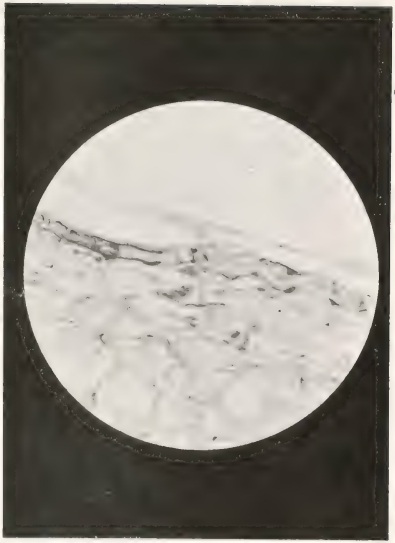


Fig. 2. A section of epidermis and cortex showing a hypha penetrating an epidermal cell. ×600 ½.

PLATE IV

Fig. 1. Three plants dead as the result of inoculation.

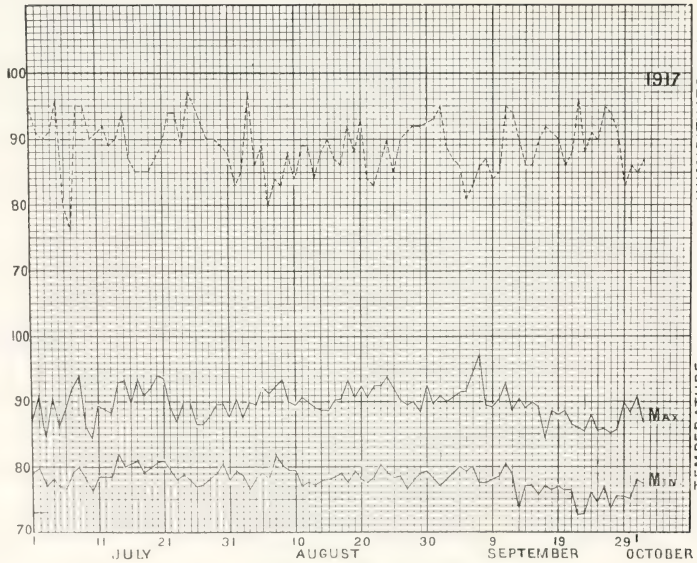
Fig. 2. Three plants of "kakya London." Left hand plant healthy; centre plant wilting as the result of inoculation; right hand plant inoculated at the base of lateral shoot.

PLATE V.

Fig. 1. Infected [illegible] plant of red stemmed variety. Realising plant wilting.

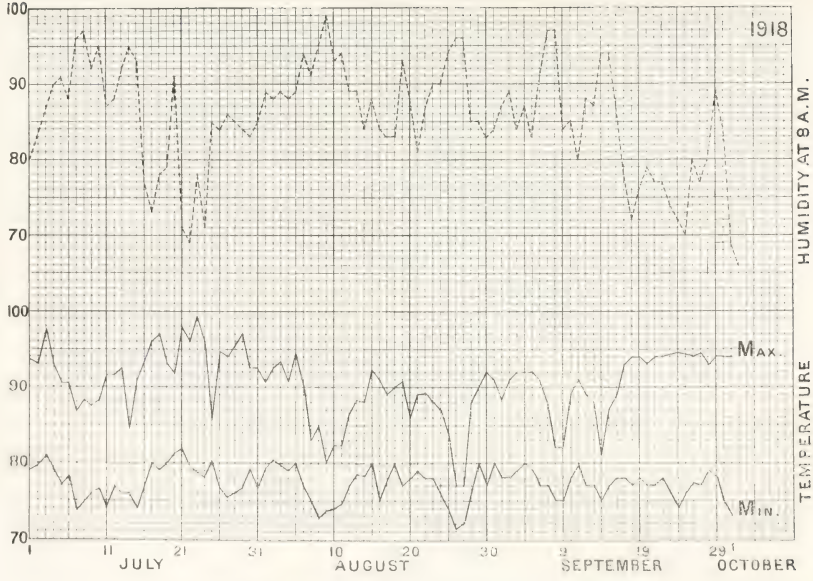
Fig. 2. Five plants of late sown jute. Four infected with *"D. Corchori* are dead. Fifth, not infected, is healthy.

PLATE VI.



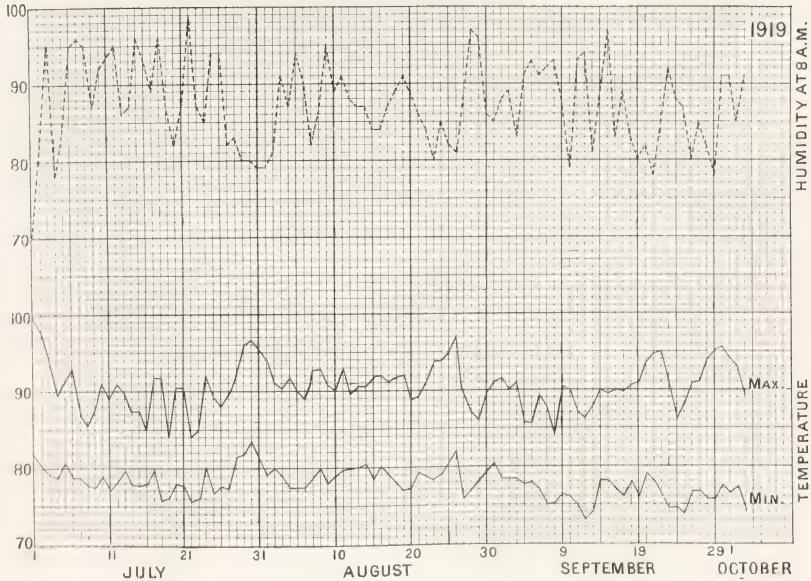
Temperature and 8 a. m. humidity curves of July, August, and September 1917.

PLATE VII.



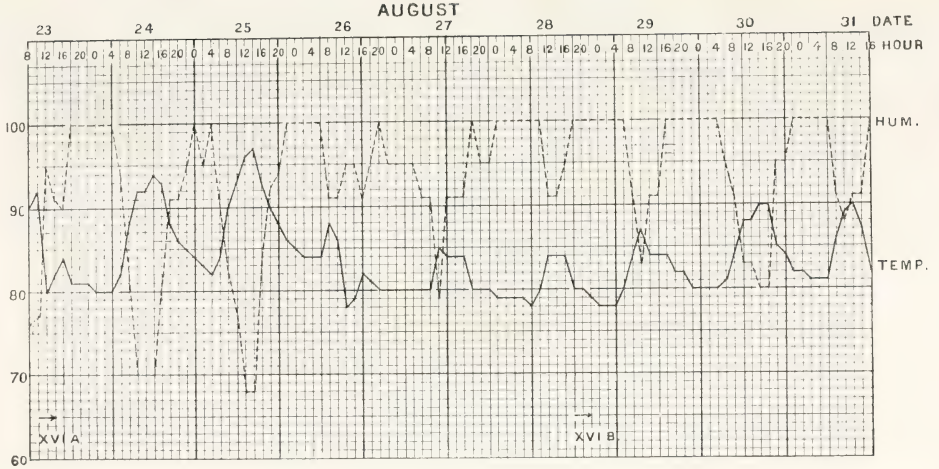
Temperature and 8 a. m. humidity curves of July, August, and September 1918.

PLATE VIII.



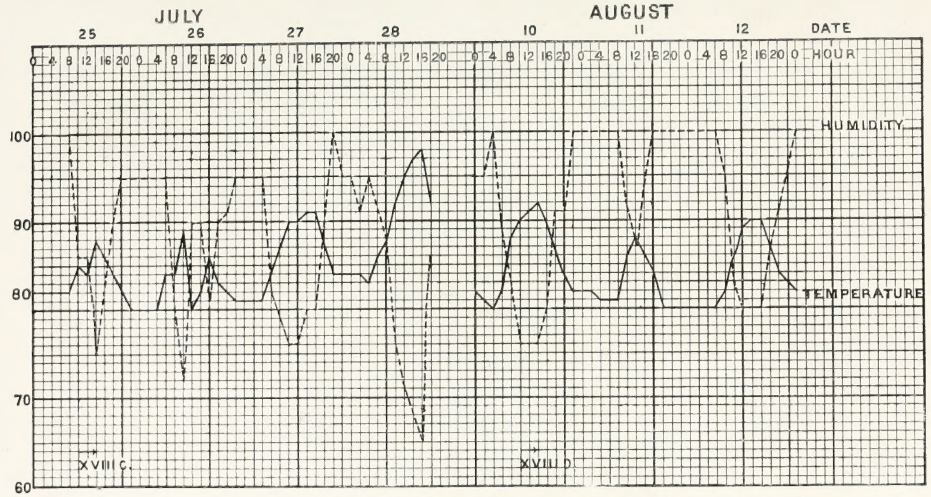
Temperature and 8 a. m. humidity curves of July, August, and September 1919.

PLATE IX.



Curves of humidity and temperature during periods of Experiments XVI *a* and *b*.

PLATE X.



Curves of humidity and temperature during periods of Experiments XVIII, *c* and *d*.

PLATE XI.